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Plasma phospholipid arachidonic acid content and calcium metabolism in idiopathic calcium nephrolithiasis

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Background. Reports of an increase in plasma and erythrocyte phospholipid arachidonic acid content and in urinary prostaglandin E_2 (PGE_2) excretion in patients with idiopathic calcium nephrolithiasis suggested their crucial role in the pathogenesis of hypercalciuria, a well-known risk factor for lithogenesis.

Methods. To confirm this hypothesis, 15 healthy subjects and 20 nephrolithiasis patients were evaluated for plasma phospholipid polyunsaturated fatty acid content and PGE_2 concentration, serum parathyroid hormone, 25 hydroxyvitamin D_3, 1,25-dihydroxyvitamin D_3, and bone-specific alkaline phosphatase levels, as well as urinary excretion of calcium, biochemical markers of bone resorption (hydroxyproline and crossLaps), and intestinal calcium absorption. Furthermore, the effect of a 30-day fish-oil diet supplementation on the previously mentioned parameters was investigated in the patients.

Results. At baseline, patients compared with controls showed higher levels of plasma phospholipid arachidonic acid content ($P = 0.002$), PGE_2 ($P = 0.0004$), serum 25-vitamin D_3 ($P = 0.001$), and 1,25-vitamin D_3 ($P = 0.001$), urinary excretion of calcium ($P = 0.001$), hydroxyproline ($P = 0.007$), and crossLaps ($P = 0.019$), as well as intestinal calcium absorption ($P = 0.03$ at 60 min). Fish oil supplementation induced a reduction in the plasma phospholipid arachidonic acid level ($P < 0.0001$), and except for serum concentrations of 25-vitamin D_3, normalized baseline blood and urinary parameters, including intestinal calcium absorption. A close correlation between plasma PGE_2 and serum 1,25-vitamin D_3 ($P = 0.004$) and between phospholipid arachidonic acid and intestinal calcium absorption ($P = 0.0002$) and calciuria ($P = 0.007$) was observed, as well as between urine excretion of crossLaps and hydroxyproline ($P < 0.0001$), crossLaps and calcium ($P < 0.0001$), and hydroxyproline and calcium ($P < 0.0001$).

Conclusions. These findings indicate that the phospholipid arachidonic acid content anomaly could represent the primary event responsible for the mosaic of metabolic and clinical alterations that are distinctive features of renal stone formers, and suggest that a common pathogenetic mechanism might account for the several forms of hypercalciuria detected in idiopathic calcium nephrolithiasis.

Key words: calciotropic hormones, hypercalciuria, lithogenesis, renal stone formers, kidney stones.

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We recently described an increased arachidonic acid (AA) level in the plasma and erythrocyte phospholipids of patients with idiopathic calcium nephrolithiasis (ICN) [1]. This finding followed previous literature reports of an alteration in urine excretion of prostaglandins E_2 (PGE_2), the main metabolite of membrane phospholipid AA, in ICN patients [2–5]. In the light of the effects of dietary-induced phospholipid AA level modifications on cellular and renal calcium transport [6, 7] and the important hypercalciuric effect of PGE_2 [5, 8, 9], we advanced that phospholipid AA content might have a crucial role in the pathogenesis of hypercalciuria, a well-known risk factor for calcium lithogenesis [1, 10, 11]. To confirm this hypothesis, in a group of ICN patients, we evaluated the relationship between plasma phospholipid AA content, calcium-regulating hormones, and intestinal, renal, and bone calcium metabolism alterations, which were observed to constitute the main causes of idiopathic hypercalciuria in calcium nephrolithiasis [12].

METHODS

Study patients

The study was conducted in 20 recurrent idiopathic calcium oxalate stone formers (male 17 and female 3; median age 36 years; range, 23 to 56) consecutively selected among those attending our outpatient clinic and fulfilling the previously described criteria for ICN [1, 13]. Fifteen healthy staff members (male 12 and female 3; median age 34 years; range, 22 to 52) served as control subjects; none had a family or personal history of nephrolithiasis. All patients and control subjects had normal renal function, sterile urine samples, and normal blood pressure; none had diabetes mellitus or hepatic, thyroid,

or immunologic disease. All gave their informed consent to the study, according to the Helsinki Declaration. No patient or control subject had been placed on any type of therapeutic regimen in the eight weeks before the study, and in particular, none was taking any lipid-lowering drug or other agent known to affect lipid metabolism. In the eight weeks preceding the study, all participants were asked to consume a diet that matched their usual caloric content and macronutrient distribution. Seventeen of the patients agreed to continue the study and received one capsule (Seacor®-SPA-Italy) containing 850 mg of ω-3 polyunsaturated fatty acid ethyl esters (fish oil) three times daily for 30 days, as previously reported [1].

Analytical procedures

At the beginning of the study, all participants fasted from 8 p.m. of the preceding evening, and the following parameters were determined: creatinine, uric acid, urea nitrogen, electrolyte concentration, lipid analysis, and the phospholipid polyunsaturated fatty acid content [1], plasma PGE_2 (PerSective Diagnostics, Cambridge, MA, USA), serum bone-specific alkaline phosphatase (BAP; Boehringer Mannheim, Milan, Italy), intact parathyroid hormone (PTH; Bio-Rad Laboratories, Milan, Italy), 25-hydroxyvitamin D_3 (25-Vit D; Buhlmann Laboratories, Allshwil, Switzerland), and 1,25-dihydroxyvitamin D_3 (1,25-Vit D; Nichols Institute, Diagnostics, San Juan Capistrano, CA, USA). Each analysis was carried out in duplicate and was expressed as the mean of two determinations. On the test day, after eight weeks of controlled diet, fasting urines were collected for two hours from 7 a.m. to 9 a.m. for assay of creatinine, urea nitrogen, calcium, and phosphorus, as well as some biochemical markers of bone turnover, including hydroxyproline (HP) (Beckman Analytical, Milan, Italy) and crossLaps (CTX; Cis Diagnostici, Vercelli, Italy). CTX measures the degradation products of type 1 collagen C-telopeptide and represents a specific marker of bone resorption [14]. All determinations were carried out in duplicate and were expressed as the mean for urine creatinine. Bone mineral density (BMD) of the lumbar spine (L2-L4) was assessed by dual-energy x-ray densitometer (Hologic QDR 1000, Boston, MA, USA). BMD values were expressed as the number of standard deviations from the mean of the normal young Caucasian population (T-score). Blood and urine parameters were re-evaluated at the end of the ω-3 fatty acid supplementation trial.

Intestinal strontium absorption test

The strontium (Sr) oral test was performed to determine intestinal calcium absorption as previously described [15] in 11 controls and in 17 patients before and after ω-3 polyunsaturated fatty acid supplementation; no food was supplied during the Sr test. Sr absorption was expressed by two different methods. The first determined the incremental area under the serum concentration-time curve (AUC at 30, 60, and 240 minutes) by the trapezoid method, and Sr absorption was expressed as $mmol/L^{-1}/minute$; the other calculated the fractional absorption (FA) of the administered dose (FA at 30, 60, 240 minutes), considering 15% of body weight as the distribution volume [15, 16].

Statistical analysis

Statistical evaluation was carried out with Student's *t*-test for paired and unpaired variables and determination of the *r* coefficient for linear correlation. The relationship between some parameters as dependent variables and others as independent variables was analyzed by a stepwise multiple regression model. Statistical significance was defined as a P value less than 0.05.

RESULTS

Findings at baseline and after fish oil supplementation are summarized in Table 1. At baseline, the patients versus control subjects showed an increase in plasma phospholipid AA content and PGE_2 values and in serum 25-Vit D and 1,25-Vit D concentrations; all the other parameters, including age, sex ratio, body mass index (BMI), and plasma lipid values, did not differ between the two groups. At the urine level, the patients presented an increased excretion of calcium, CTX, and HP. Moreover, the patients showed an increase in intestinal absorption of Sr that was significant at 30 and 60 minutes (both expressed as FA and AUC) and at 240 minutes (expressed as AUC; Table 2). Mean T-scores in the two groups showed no significant difference; six patients had the T-scores that were a little lower, but not more than 10%.

The effects of fish oil supplementation are reported in Table 1. This regime induced a reduction in the plasma phospholipid content of linoleic and AA and an increase in eicosapentaenoic and docosahexaenoic acids. Moreover, the fish oil supplementation was able to modify the blood and urine parameters that were altered at baseline. It lowered calcium-regulating hormones, PGE_2 and 1,25-Vit D, but not 25-Vit D values, and reduced intestinal Sr absorption (Table 2), urine calcium excretion (Fig. 1), and the biochemical markers of bone remodeling, such as serum BAP and urine HP and CTX. No correlation was observed between the plasma phospholipid AA content and PGE_2 values ($r = 0.14$). In contrast, a direct significant correlation between plasma PGE_2 and serum 1,25-Vit D values emerged (Fig. 2), as well as a close correlation between the phospholipid AA level and intestinal Sr absorption (Table 3).

Moreover, a direct correlation between urine excretion of CTX and HP (Fig. 3), CTX and calcium (Fig. 4), and HP and calcium (Fig. 5), and between plasma phospholipid AA content and calciuria (Fig. 6) was observed. Stepwise multiple analysis showed that plasma phospho-

Table 1. Biochemical parameters of control subjects and patients before and after fish oil supplementation

	Controls	Patients		*P* values[a]	*P* values[b]	*P* values[c]
		Before	After			
Plasma fatty acid %						
C18:0 (Stearic)	13.50 ± 0.80	13.73 ± 1.29	14.18 ± 0.99	0.5474	0.0627	0.0406
C18:2 n-6 (Linoleic)	25.75 ± 2.55	24.67 ± 2.39	21.35 ± 3.02	0.2076	0.0001	0.0001
C20:4 n-6 (Arachidonic)	10.04 ± 0.91	11.17 ± 1.08	9.58 ± 1.17	0.0024	<0.0001	0.2336
C20:5 n-3 (EPA)	0.63 ± 0.16	0.77 ± 0.29	4.88 ± 0.88	0.1219	<0.0001	<0.0001
C22:6 n-3 (DHA)	3.82 ± 0.68	3.37 ± 0.94	6.44 ± 1.02	0.1177	<0.0001	<0.0001
Plasma phospholipids *mg/dL*	200.10 ± 25.10	201.40 ± 30.90	203.44 ± 28.02	0.8943	0.6656	0.7259
Plasma PGE_2 *ng/L*	111.73 ± 37.66	163.94 ± 39.24	95.82 ± 48.23	0.0004	<0.0001	0.3109
Serum PTH *ng/L*	32.93 ± 10.53	31.10 ± 11.35	37.41 ± 15.99	0.6290	0.0951	0.3636
Serum 25-vitamin D *nmol/L*	46.96 ± 11.71	62.55 ± 14.24	65.29 ± 20.27	0.0015	0.6038	0.0043
Serum 1,25 vitamin D *pmol/L*	44.45 ± 10.23	60.15 ± 15.58	44.33 ± 21.67	0.0018	0.0085	0.9848
Serum 25-vitamin D/1,25-vitamin D	1.15 ± 0.40	1.28 ± 0.53	2.06 ± 1.49	0.4355	0.0228	0.0291
Serum Calcium *mmol/L*	2.34 ± 0.07	2.29 ± 0.11	2.31 ± 0.09	0.1144	0.6049	0.3131
Serum Phosphorus *mmol/L*	1.29 ± 0.15	1.19 ± 0.18	1.22 ± 0.23	0.1155	0.6632	0.3300
Serum BAP *U/L*	28.10 ± 10.50	30.23 ± 16.34	26.94 ± 16.21	0.6623	0.0025	0.8146
Urinary urea *mmol/mmol Cr*	12.84 ± 2.45	13.91 ± 3.65	13.01 ± 4.00	0.3345	0.7088	0.8837
Urinary calcium *mmol/mmol Cr*	0.08 ± 0.03	0.13 ± 0.05	0.08 ± 0.04	0.0011	0.0348	0.9342
Urinary Phosphorus *mmol/mmol Cr*	0.35 ± 0.05	0.28 ± 0.06	0.31 ± 0.15	0.0017	0.1361	0.3802
Urinary CTX *μg/mmol Cr*	259.22 ± 57.76	323.82 ± 88.24	217.45 ± 96.58	0.0192	0.0179	0.1543
Urinary HP *μmol/mmol Cr*	12.00 ± 5.21	18.01 ± 6.96	11.12 ± 7.66	0.0078	0.0395	0.7102

Plus-minus values are means ± SD. Abbreviations are: P-Fatty acid, the percentage of fatty acids in plasma phospholipids; EPA and DHA, eicosapentaenoic and docosahexaenoic acids; BAP serum bone-specific alkaline phosphatase; CTX, crossLaps; HP, hydroxyproline.

[a] Comparison between patients (before) and control subjects (unpaired *t*-test)

[b] Comparison between patients before and after treatment (paired *t*-test)

[c] Comparison between patients (after) and control subjects (unpaired *t*-test)

Table 2. Strontium (Sr) oral load test in control subjects and patients before and after fish oil supplementation

	Controls	Patients		*P* values[a]	*P* values[b]	*P* values[c]
		Before	After			
Sr-FA 30 min	13.98 ± 6.14	18.92 ± 5.91	12.53 ± 5.29	0.0420	0.0002	0.5112
Sr-FA 60 min	22.84 ± 7.12	29.31 ± 8.00	24.75 ± 6.85	0.0380	0.0040	0.4836
Sr-FA 240 min	27.55 ± 10.72	30.87 ± 8.10	30.38 ± 10.38	0.3589	0.7951	0.4911
Sr-AUC 30 min	0.42 ± 0.19	0.57 ± 0.17	0.38 ± 0.16	0.0406	0.0002	0.5102
Sr-AUC 60 min	1.54 ± 0.53	2.03 ± 0.60	1.51 ± 0.48	0.0354	0.0001	0.8736
Sr-AUC 240 min	10.68 ± 3.57	12.95 ± 3.84	11.51 ± 3.29	0.1279	0.0182	0.5346

Plus-minus values are means ± SD. Sr-FA and Sr-AUC denote the intestinal Sr absorption expressed by two ways (**Methods**).

[a] Comparison between patients (before) and control subjects (unpaired *t*-test)

[b] Comparison between patients before and after treatment (paired *t*-test)

[c] Comparison between patients (after) and control subjects (unpaired *t*-test)

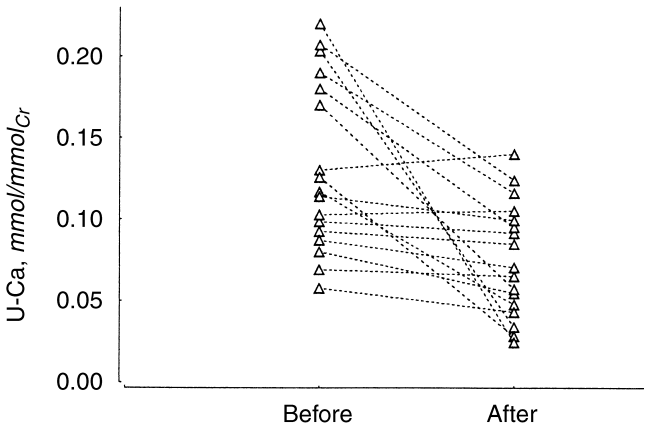

Fig. 1. Effects of fish oil administration on urine calcium excretion ($P = 0.35$).

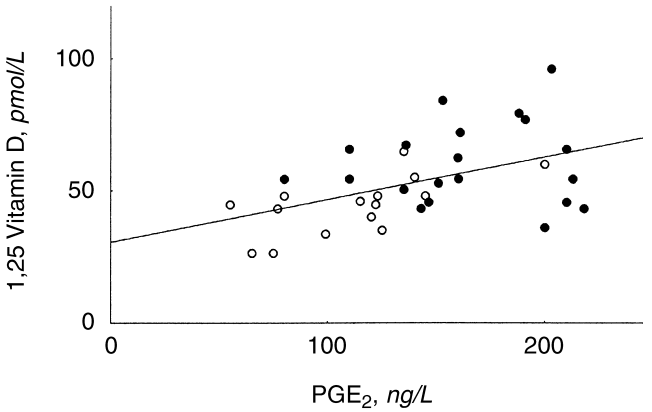

Fig. 2. Relationship between plasma prostaglandin E_2 (PGE_2) and serum 1,25-vitamin D_3 (1,25-Vit D) concentration in control subjects (○) and patients (●) ($r = 0.47$, $P = 0.0044$).

Table 3. Relationships between plasma phospholipid arachidonic acid content (P-AA) and intestinal strontium absorption rate expressed by fractional absorption and area under the serum concentration curve (AUC) procedures (**Methods** section)

	r	*P* values
P-AA/Sr-FA 30 min	0.41	0.0292
P-AA/Sr-FA 60 min	0.65	0.0002
P-AA/Sr-FA 240 min	0.66	0.0001
P-AA/Sr-AUC 30 min	0.41	0.0292
P-AA/Sr-AUC 60 min	0.54	0.0028
P-AA/Sr-AUC 240 min	0.68	0.0001

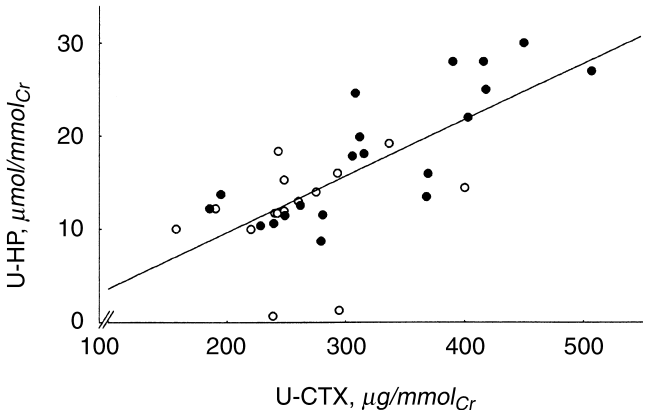

Fig. 3. Relationship between urine excretion of crossLaps (CTX) and hydroxyproline (HP) in control subjects (○) and patients (●) ($r = 0.72$, $P < 0.0001$).

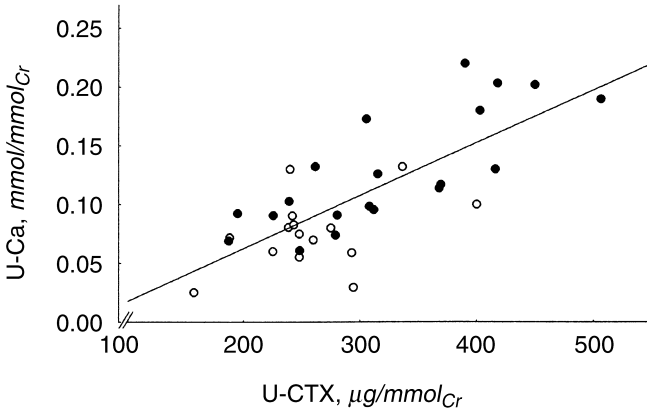

Fig. 4. Relationship between urine crossLaps (CTX) and calcium excretion in control subjects (○) and patients (●) ($r = 0.75$, $P < 0.0001$).

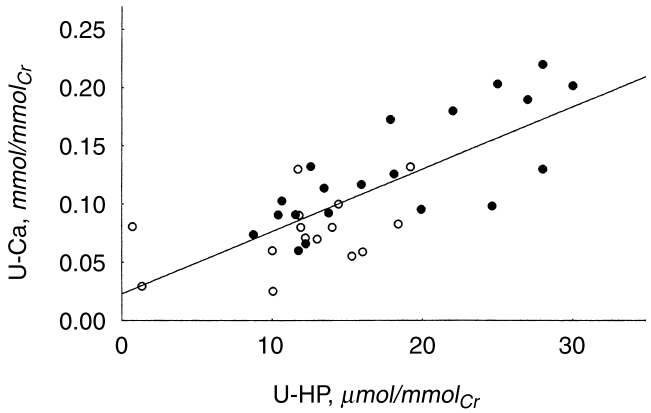

Fig. 5. Relationship between urine HP and calcium excretion in subjects (○) and patients (●) ($r = 0.72$, $P < 0.0001$).

lipid AA content and serum 1,25-Vit D concentration were the only statistically significant variables influencing intestinal Sr absorption at 60 minutes (Table 4), and that the calciuria was best explained by urine CTX and HP excretion, as well as intestinal calcium absorption (Table 5).

DISCUSSION

The clinical and biochemical profiles of the patients who constituted the basis of our study showed the typical features of idiopathic calcium renal stone formers. Indeed, besides the hypercalciuria, we observed an alteration in calcium-regulating hormones, such as serum levels of calcidiol and calcitriol, and an increase in intestinal calcium absorption and bone turnover, as documented by the Sr test and the urinary excretion of biochemical markers of bone resorption (CTX and HP).

Hypercalciuria is the most common metabolic abnormality in ICN. It appears to reflect a heterogeneous entity and to result from a variety of causes, including dietary calcium excess, intestinal calcium hyperabsorption, calcium renal leak, and bone loss [12]. The problem is whether the several types of idiopathic hypercalciuria, including the absorptive, renal, and resorptive forms, reflect a spectrum of independent calcium disorders or an epiphenomenon of a primary, as yet undefined generalized disturbance of calcium metabolism [17–19]. As 1,25-Vit D plays a central role in calcium metabolism at the intestinal, renal, and bone levels, a disorder in its pathway was a most attractive hypothesis to explain the different forms of hypercalciuriua. Indeed, increased serum 1,25-Vit D levels have been observed in patients with hypercalciuria [17, 20–22] and have been confirmed by our study. Considering the selection criteria we used, the difference in 1,25-Vit D levels between patients and controls in our study did not depend upon different sunlight exposure times or other environmental factors. Moreover, calcitriol synthesis might be activated perhaps via a yet uncharacterized disorder in phosphate metabolism or, more simply, a mechanism(s) that up-regulates receptors for calcitriol. Both processes increase intestinal calcium absorption and urinary calcium excretion, and probably bone turnover as well [23–26]. However, most studies in this field have not yet reached definitive conclusions. In particular, it is not clear whether the disorder in the 1,25-Vit D pathway is a primary one or secondary to some other calcium metabolism defect. In our opinion,

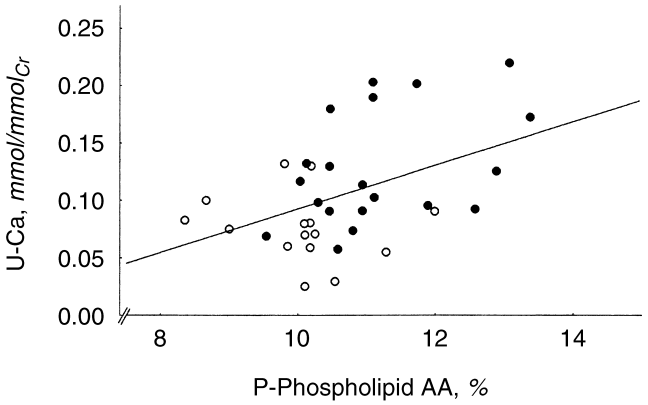

Fig. 6. Relationship between plasma phospholipid arachidonic acid (AA) content and calciuria in control subjects (○) and patients (●) (r = 0.44, P = 0.0075).

Table 4. Multivariate regression analysis of the relationship between intestinal strontium absorption rate (Sr-FA 60 min) and other biochemical variables

Variables	Statistics β	SE	P values
Sr-FA 60 min			
P-AA	0.666	(0.136)	0.0001
S-1,25 Vit D	0.351	(0.121)	0.0239
	F = 14.440	$R^2 = 0.53$	$P < 0.0001$

P-AA denotes the plasma phospholipid arachidonic acid content. The analysis was carried out in 28 subjects (11 controls and 17 patients) and is the result of a stepwise multiple regression by excluding the less significant variables. β denotes standardized regression coefficient.

Table 5. Multivariate regression analysis of the relationship between urine calcium excretion and other biochemical variables

Variables	Statistics β	SE	P values
U-Ca			
U-CTX	0.452	(0.135)	0.0021
U-HP	0.407	(0.135)	0.0052
Sr-FA 60 min	0.265	(0.014)	0.0082
	F = 27.844	$R^2 = 0.73$	$P < 0.0001$

The analysis was carried out in 28 subjects (11 controls and 17 patients) and is the result of a stepwise multiple regression by excluding the less significant variables. Abbreviations are: CTX, crossLaps; HP, hydroxyproline; Sr-FA, 60 minutes of intestinal Sr absorption; β, standardized regression coefficient.

the present study provides new and important insights into the pathophysiology of idiopathic hypercalciuria because it confirms our previous hypothesis of a causal link between membrane phospholipid AA levels and the calcium-related abnormalities frequently occurring in ICN patients [1, 10, 11]. This statement is supported by the results observed at baseline and is reinforced by the effects of polyunsaturated fatty acid administration. Indeed, we found an increase in the plasma phospholipid AA content, which agrees well with our previous finding in another group of nephrolithiasic patients [1]. As expected [1, 27], and also confirming good dietary compliance, fish oil supplementation was able to induce a reduction in the plasma phospholipid AA level. Moreover, it demonstrated a hypocalciuric effect that is in agreement with previous reports [1, 28]. This regimen also induced a reduction in PGE_2 (the main metabolite of AA) and calcitriol values, as well as in intestinal Sr absorption, and a decrease in bone turnover, as documented by the reduction in biochemical markers of bone resorption (urinary HP and CTX excretion) and bone formation (serum BAP values).

Whatever the mechanism underlying the rise in the phospholipid AA proportion, we suggested that this anomaly could play a crucial role in the pathogenesis of idiopathic hypercalciuria through different actions. Indeed, an increase in phospholipid AA content determines a cascade of metabolic effects on calcium homeostasis leading to hypercalciuria, essentially because of a direct action of AA as second messenger on cellular calcium transport [29] or through the PGE_2/1,25-Vit D pathway. PGE_2, the main metabolite of AA, is known to modulate renal calcium handling by inhibiting the Na/K/2 Cl cotransporter, whose activity is important in driving calcium tubular reabsorption [5, 8] or by other mechanisms [9]. Moreover, the effects of PGE_2 on the activity of 1,α hydroxylase, the rate-limiting step in the formation of calcitriol, are well known [9], and seem to be confirmed by the direct correlation that emerged between PGE_2 and 1,25-Vit D values (Fig. 1) and the results of fish oil supplementation, which showed a significant decrease in 1,25-Vit D levels, but not in those of its precursor 25-Vit D, leading to an increase in the calcidiol/calcitriol ratio. Excess calcitriol production was shown to induce intestinal calcium hyperabsorption, and reduced calcium tubular reabsorption [9]. Interestingly, a positive correlation was observed between plasma phospholipid AA level and intestinal Sr absorption (Table 3). Moreover, multiple regression analysis disclosed that the plasma phospholipid AA content and serum 1,25-Vit D levels were the only statistically significant and independent variables influencing intestinal Sr absorption (Table 4), thus suggesting a direct and independent effect of AA, besides the PGE_2/1,25-Vit D mechanism, on intestinal calcium transport.

Regarding the possible link between phospholipid AA values and bone remodeling, it is noteworthy that PGE_2 and 1,25-Vit D, besides their effects on renal and intestinal calcium handling, also play a role in stimulating bone resorption and could constitute an additional source of urinary calcium in ICN [30]. The observations of primary or secondary high bone turnover in some hypercalciuric renal stone formers were confirmed by the present data. Indeed, we found an increase in specific biochemical markers of bone resorption, a positive correlation between urine excretion of calcium, CTX, and HP (Figs. 3 and 4), and a simultaneous reversal effect of dietary fish

oil administration on calciotropic hormones, biochemical markers of bone turnover, and urine calcium excretion. Calcitriol is a potent stimulator of osteoclast formation in vitro as well in vivo [31–33], while PGE_2 is an important factor of bone resorption directly or by the activation of 1,25-Vit D synthesis [34, 35]. On the other hand, it was reported that calcitriol may induce interleukin-1 (IL-1) osteoblastic receptor expression [36], and AA can modulate the synthesis of cytokines and growth factors [37, 38], well-known important local factors that are also involved in bone remodeling [39–41]. IL-1 and tumor necrosis factor-α (TNF-α) are among the most powerful stimulators of bone resorption and are well-recognized inhibitors of bone formation. The notion of a link between AA and bone turnover seems to be confirmed by previous findings that dietary fish oil supplementation reduced bone turnover in an experimental animal model [42] and decreased IL-1, IL-6, and TNF-α synthesis by circulating monocytes [40, 43]. Moreover, preliminary results from our laboratory demonstrated a specific effect of AA on the genetic expression of IL-1, IL-6, and TNF-α in a human osteoblastic cell line [44].

On the whole, the results of the present study seem to confirm the hypothesis of a close association in ICN patients between plasma phospholipid AA levels, calcium-regulating hormones, and urinary calcium excretion. A logical interpretation of our observations is that a higher phospholipid AA content would induce an increased calciotropic hormone concentration, thus leading to an increased intestinal calcium absorption, a decreased renal tubular calcium reabsorption, and an increased calcium bone loss, all mechanisms that have been observed to contribute to hypercalciuria in ICN. This idea is supported by multiple regression analysis that showed that urinary calcium excretion was well explained by the urine HP and CTX excretion and by intestinal calcium absorption (Table 5), thus suggesting that calciuria ultimately reflects the net rates of either intestinal calcium absorption or bone resorption, processes that are integrated by the calciotropic hormones, and perhaps other systems. In this setting, it is interesting to note that urinary calcium excretion was positively correlated with the plasma phospholipid AA level (Fig. 5).

One could speculate whether the phospholipid AA level anomaly really can explain the mosaic of metabolic and clinical alterations that are distinctive features of ICN patients, such as serum PGE_2 and 1,25-Vit D levels, kidney, intestine, and bone calcium metabolism, and several forms of idiopathic hypercalciuria [10]. In our opinion, further investigations in a larger number of hypercalciuric patients, classified according to the conventional criteria for differentiating hypercalciuria, will be important for recognizing in this anomaly, the primary event responsible for idiopathic hypercalciuria. Nonetheless, the results of the present study seem to reinforce our opinion and that of others [17, 18, 45] that, despite differences in the clinical presentation, a common pathogenetic mechanism may account for the several subtypes of idiopathic hypercalciuria in ICN, which represents an epiphenomenon of a systemic disturbance in calcium metabolism.

Finally, in addition to the investigations of the yet unknown origin of the anomalous phospholipid AA level, to which genetic and nutritional/environmental factors seem to contribute [1], the positive and long-term effects of polyunsaturated fatty acid administration on the metabolic and clinical features of ICN patients and the natural history of this renal disease need further clinical verification. In this regard, it is worth noting that Eskimos, who eat a ω-3 fatty acid-rich diet and seem to have a very low desaturase activity [46], the rate-limiting step in the biosynthetic pathway of highly unsaturated fatty acids [47], do not generally develop nephrolithiasis. Moreover, in studies of Japanese stone formers, it was found that the prevalence of nephrolithiasis since the Second World War correlated positively with the increase not only in protein, but also in animal fat [48, 49]. On the other hand, it is known that ICN emerged as a disease entity in Western countries at the beginning of this century, with an expanding societal weight running parallel with affluency.

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